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Award Number: DAMD17-01-1-0571

TITLE: Heat Shock Protein 27 Inhibits Apoptosis by Binding Cytochrome c

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REPORT DATE: June 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2002	Annual (1 Jun 01 - 31 May 02)

4. TITLE AND SUBTITLE
Heat Shock Protein 27 Inhibits Apoptosis by Binding Cytochrome c

5. FUNDING NUMBERS
DAMD17-01-1-0571

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The purpose of this research project is to investigate the interaction between heat shock protein 27 (hsp27) and cytochrome c in the inhibition of apoptosis. The scope of the study will include: measuring the binding of hsp27to cytochrome c in vivo, determining why hsp27 binds to cytochrome c and determining the fate of the hsp27:cytochrome c complex. Major findings to date include: cellular survival cures to cytochrome c and molecular chaperone assays with hsp27 and citrate synthase and alpha-glucosidase. A no-cost one year time extension was granted to complete the goals of the project.

14. SUBJECT TERMS
breast cancer

15. NUMBER OF PAGES
5

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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INTRODUCTION:

This annual report is for the period June 2001 – May 2002. The objectives of the purposed study are to (1) measure the binding of hsp27 to cytochrome c in vivo, (2) determine why hsp27 binds to cytochrome c and (3) determine the fate of the hsp27:cytchrome c complex. We have made significant progress on the stated objectives but have not yet achieved them, so we asked for and received a one year, no-cost, time extension to complete the objectives.

BODY:

The first objective, measure the binding of hsp27 to cytochrome c in vivo, is progressing well. We have made extracts of cells induced to undergo apoptosis. We have also successfully conducted Western blot analysis of in vitro immunoprecipiated samples of hsp27 and cytochrome c. We are now ready to begin the immunoprecipiation of the in vivo samples. To demonstrate that cytochrome c can interact with hsp27 in vivo, we have added cytochrome c to whole cells and determined that cells that constitutively express hsp27 survive at a much greater frequency then cells that did not express hsp27. We believe (and are in the process of demonstrating) that cytochrome c enters the cell and triggers apoptosis.

The second objective, determine why hsp27 binds to cytochrome c is in progress. We have begun using a DHFR refolding model and have determined the conditions that are necessary to thermally denature DHFR. We have also used citrate synthase and alpha-glucosidase as model systems as well.

The last objective, determine the fate of the hsp27:cytochrome c complex, is also showing good progress. We have initially focused on using a chromatography approach to help identify the hsp27:cytochrome c complex. We are using the apoptosome as a model system to determine if hsp27 can prevent its formation. We have determined the optimum conditions for the column and are now ready to begin a time course study.

KEY RESEARCH ACCOMPLISHMENTS:

Cellular Survival Curves to Cytochrome c.
Hsp27 Molecular Chaperone assays with Citrate Synthase and Alpha-Glucosidase
Determination of Column Conditions for Apoptosome Detection

REPORTABLE OUTCOMES:

Scientific talk "The role of heat shock proteins in the inhibition of apoptosis" given at the 2002 Radiation Research Society Meeting in Reno, NV that outlined our progress on this project.

Poster session "Heat Shock Protein 27 Inhibits Apoptosis in Human Breast Cancer cells by Binding to Cytochrome c" given at the 2002 Era of Hope U.S. Army Breast Cancer Research Conference in Orlando, Fl., that outlines our progress to date.

CONCLUSIONS:

More time is needed to achieve the objectives. We believe that we will be able to achieve the three goals by the end of the time extension. The data generated by these studies should be sufficient for two publications as well as a competitive grant application to the U.S. Army to extent these studies.

REFERENCES: None

APPENDICES: None